U.S. DEPARTMENT OF COMMERCE
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PB-268 118

Biological Assessment of Continuous Exposure to Tritium and Lead in the Rat

Health Effects Research Lab, Research Triangle Park, N C

1976

PB 268 118

EPA-600/1-76-061
JOURNAL ARTICLE

Reprint from

"BIOLOGICAL
AND ENVIRONMENTAL EFFECTS
OF LOW-LEVEL RADIATION"

VOL.II

International Atomic Energy Agency, Vienna,
IAEA-SM-202/305, 65-78, 1976

INTERNATIONAL ATOMIC ENERGY AGENCY
VIENNA, 1976

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SPRINGFIELD, VA. 22161

MS-75-032

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA-600/1-76-063	2. JOURNAL ARTICLE
3. RECIPIENT'S ACCESSION NO.	
4. TITLE AND SUBTITLE: Biological Assessment of Continuous Exposure to Tritium and Lead in the Rat	5. REPORT DATE
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S): D.F. Cahill, L.W. Reiter, J.A. Santolucito, G.I. Rehnberg, M.E. Ash, M.J. Favor, S.J. Bursian, J.F.Wright and J.W. Laskey	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS: Health Effects Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, N.C. 27711	10. PROGRAM ELEMENT NO. 1FA628
	11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS: Same	13. TYPE OF REPORT AND PERIOD COVERED
	14. SPONSORING AGENCY CODE EPA/600/11

15. SUPPLEMENTARY NOTES
In: BIOLOGICAL AND ENVIRONMENTAL EFFECTS OF LOW-LEVEL RADIATION, Vol. II, International Atomic Energy Agency, Vienna, IAEA-SM-202/305, 65-78, 1976

16. ABSTRACT

A broad investigation of the effects of simultaneous exposure to two potentially synergistic environmental pollutants, tritiated water (HTO) and lead, was conducted. Sprague-Dawley rats were continuously exposed to HTO and/or Pb in drinking water from conception of the F_1 through adulthood of the F_2 generation. A 12-cell exposure matrix was used employing HTO at levels calculated to provide approximately 0 - 300 mrad/d whole-body irradiation and Pb levels of 0 or 50 ppm in drinking water. Observations were made on the reproductive capacity of the F_1 generation and the effects of lifetime parental exposure to HTO and/or lead on the F_2 neonates. The effects of single and combined exposures on the development and function of the central nervous system, some brain catecholamine levels and electroencephalogram patterns were also examined in both generations. The results indicate that, in both generations, continuous HTO exposures as low as 3 mrad/d delayed development of righting reflexes in young rats. 30 mrad/d additionally depressed the spontaneous activity of adult male rats. Continuous exposure to 5 ppm lead produced similar effects on righting reflex development and adult spontaneous activity. The relative brain weight of F_2 neonates was decreased after lifetime parental exposure to 300 mrad/d or 5 and 50 ppm lead. Chronic lead exposure also appears to induce hypercorticalism and increase preimplantation deaths in F_1 dams. These effect responses to both HTO and lead were less than additive in their interactive effects on the parameters measured.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
lead tritium rats laboratory animals irradiation		06 T 06 F 06 R

18. DISTRIBUTION STATEMENT: RELEASE TO PUBLIC	19. SECURITY CLASS (This Report): UNCLASSIFIED	21. NO. OF PAGES: 15
	20. SECURITY CLASS (This page): UNCLASSIFIED	22. PRICE: A02 - A01

EPA Form 2220-1 (9-73)

IAEA-SM-292/85

BIOLOGICAL ASSESSMENT OF CONTINUOUS EXPOSURE TO TRITIUM AND LEAD IN THE RAT

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Abstract

BIOLOGICAL ASSESSMENT OF CONTINUOUS EXPOSURE TO TRITIUM AND LEAD IN THE RAT.

A broad investigation of the effects of simultaneous exposure to two potentially synergistic environmental pollutants, tritiated water (HTO) and lead, was conducted. Sprague-Dawley rats were continuously exposed to HTO and/or Pb in drinking water from conception of the F_1 through adulthood of the F_1 generation. A 2-cell exposure matrix was used employing HTO activities calculated to provide approximately 3 - 300 mrad/d whole-body irradiation and Pb levels of 5 or 50 ppm in drinking water. Observations were made on the reproductive capacity of the F_1 generation and the effects of lifetime parental exposure to HTO and/or lead on the F_1 neonates. The effects of single and combined exposures on the development and function of the central nervous system, some brain catecholamine levels and electroencephalogram patterns were also examined in both generations. The results indicate that, in both generations, continuous HTO exposures as low as 3 mrad/d delayed development of righting reflexes in young rats; 30 mrad/d additionally depressed the spontaneous activity of adult male rats. Continuous exposure to 5 ppm lead produced similar effects on righting reflex development and adult spontaneous activity. The relative brain weight of F_1 neonates was decreased after lifetime parental exposure to 300 mrad/d or 5 and 50 ppm lead. Chronic lead exposure also appears to induce superovulation and increase preimplantation deaths in F_1 dams. Dose-effect responses to both HTO and lead were less than additive in their interactive effects on the parameters measured.

INTRODUCTION

In an attempt to contribute information on the effects of simultaneous exposure to two potentially synergistic environmental pollutants we have chronically administered tritiated water (HTO) and lead (Pb) to rats. HTO and Pb were selected because they are ubiquitous in the environment; technological advances have significantly increased their environmental levels; both are transplacentally transferred [1,2] and can affect intrauterine development [3,4]. Further, exposure to radiation or Pb in utero can produce postnatal neurological and reproductive dysfunction [3-6]. The chronic exposure levels employed were 0.01 - 1.0 μCi HTO/ml body water (3-300 millirads/day) and 5 and 50 ppm of Pb in drinking water. These are, respectively, 2-200 times MPC_w for an individual in the general population [7] and 100-1000 times the U.S. Drinking Water Standards [8].

MATERIALS AND METHODS

Male and female Sprague-Dawley rats (Blue Spruce Farms, Altamont, NY) ages 50-60 days, were randomly assigned to one of three treatment groups: 0, 5 or 50 ppm Pb (acetate) administered in deionized drinking water for 40 days prior to mating. When a positive sperm determination was made

these P_0 females were assigned to one of four HTO groups: 0, .01, .10 or 1.0 uCi/ml and injected I.P. with sufficient HTO to establish the desired body-water activity. HTO activities in body water were maintained by ad libitum access to HTO drinking water supplies. The result was a 12-cell exposure matrix of pregnant P_0 rats exposed to HTO only, Pb only or combinations of HTO and Pb.

F_1 progeny were thus continuously exposed to the HTO and/or Pb regimens from conception through gestation and postnatal life. At 100 days, the F_1 rats were mated and some F_2 litters were delivered by Caesarotomy at full term for observation of the F_2 neonates. P_0 and F_1 dams and the F_2 female offspring were removed from the study at weaning. F_1 and F_2 male rats continued to be exposed until 180 days of age. Naturally delivered litters in both the F_1 and F_2 generations were reduced to 8 pups per dam on day 1 and weaned on day 21.

OBSERVATIONS ON F_2 NEONATES

Following delivery of the F_2 by Caesarotomy, the ovaries from 6-8 dams per dose group were removed for corpora lutea examination and the uteri examined for resorption sites. F_1 maternal blood, liver and kidneys were taken for Pb analyses. Litter size was recorded, the fetuses sexed, examined for topographical anomalies and weighed. The brain, heart, lung, liver, kidneys and spleen from two males and two females in each litter were removed, weighed and frozen for subsequent Pb analysis. Pooled blood samples were obtained from the remaining pups in each litter for Pb determination.

OBSERVATIONS ON ADULT F_1 AND F_2 MALES

Six F_1 and F_2 males per treatment group were sacrificed at 180 days of age and body weight recorded. Blood, brain, liver, kidneys, testes and femur were removed, weighed and frozen for subsequent lead analysis. Catecholamine analyses were also performed on whole brain. Complete gross and histopathologic evaluations were performed on these males.

LEAD AND TRITIUM ANALYSIS

HTO activities in each batch of drinking water were determined by liquid scintillation counting. The average body-water HTO activity for the 2-3 rats in each cage was determined from composite 24-hour urine samples collected biweekly.

Pb determinations were done with a Perkin-Elmer model 306 atomic absorption spectrophotometer (AAS) with a three-slot burner head and deuterium background corrector. The Pb content of each batch of control and Pb water supplies was verified by AAS analysis. Blood and tissue Pb concentrations were determined by the Delves cup method of Fernandes and Kahn [9]. Bone and food samples were analysed by the method of Yeager et al.[10]. Adequacy of the analytical procedures was evaluated by assaying a National Bureau of Standards Bovine Liver (No. 1577) by the methods outlined above.

Delta aminolevulinic acid (ΔALA) was measured in urine by the method of Marver et al.[11].

DEVELOPMENTAL TESTING

Five pups from each of 4-6 litters per treatment group in both the F_1 and F_2 generations were tested for the ages at which the startle response, the righting reflex and eye opening developed. The startle response was evoked by a click (generated by a toy clicker) sounded immediately behind the head of an animal held suspended by the nape of the neck. A positive response consisted of a muscle jerk in any of the extremities.

The righting reflex was elicited by holding the rat upside down by the nape of the neck and the tail and dropping it onto wood shavings from a height of approximately 30 cm. A response was considered positive if the animal righted in the air and landed on all four feet.

Age at eye opening was recorded upon complete opening of both palpebral fissures.

LOCOMOTOR ACTIVITY

The locomotor activity of adult F_1 and F_2 males was recorded at approximately 105 days of age in a residential maze as previously described [12]. Groups of three littermates from 6 litters per dose group in both generations were tested in the maze for a period of 23 hours per day for 4 consecutive days. The mazes were housed in a sound-attenuated room maintained on a 12-hour light/dark cycle beginning at 0100am. Food and water were available ad libitum. Activity counts were recorded utilizing a Xerox mini-computer. Data obtained on days 2-4 were averaged to obtain mean daily activity levels for 6 litters. The average of the 6 litter averages was used to compute treatment means.

CATECHOLAMINE ASSAYS

In both the F_1 and F_2 generations, 6-8 males and females from each dose group (28 days old) were decapitated and 5-10 adult male rats (180 days old) were killed by pentobarbital injection and decapitated. Whole brains were removed, weighed and immediately frozen on dry ice. Catecholamines were isolated from whole brain on aluminum oxide following the method of Anton and Sayre [13]. Norepinephrine (NE) was assayed fluorometrically according to von Euler and Lishajko [14] and dopamine (DA) by the method of Carchas, Erdelyi and Angwin [15].

ELECTROENCEPHALOGRAMS (EEG)

EEGs of 180-day-old F_1 and F_2 male rats were measured. Rats were anesthetized with sodium pentobarbital (40 mg/kg) i.p. After loss of the blink reflex and attainment of abdominal breathing, platinum needle electrodes were placed subcutaneously in the scalp at mid-frontal (MF) and right and left occipito-parietal (RO and LO) locations. Bipolar recordings were made from the MF x RO lead-pair on a Beckman Dynograph. Simultaneously, the amplified signal was subjected to interval analysis using a DEC Lab-8 computer. Two 2-1/2 minute segments of anesthetized EEG were machine-processed to provide the zero-potential crossover (ZPC) rate per 5-second epoch, and the percent abundance of waveforms in β (13-90 Hz), α (7.7-12.5 Hz), θ (4-<8 Hz) and δ (<4 Hz) frequency classes.

STATISTICAL ANALYSIS

HTO or Pb effects were analysed by a one-way analysis of variance (ANOVA) and a t-test. HTO/Pb interaction was tested by a two-way ANOVA with the null hypothesis that the interaction was additive and linear.

RESULTS

Throughout the experiment the HTO activities and Pb levels in the drinking water were maintained within ± 10% of the design levels. Further, the Pb levels in deionized drinking water were < 0.01 ppm, in ambient air < 0.1 μg/M³, and in rat food an average of 0.9 ppm.

HTO had no effect on the growth of the F_1 or F_2 although a transient body-weight deficit was observed at ages 9-15 days in the F_2 generations. In both generations, rats exposed to Pb only or HTO/Pb were not significantly different in body weight from controls at any age nor did exposures to Pb produce overt signs of Pb intoxication. Hematocrits of adult F_1 rats were indistinguishable from controls but the ΔALA level in the urine of adult F_1 rats exposed to 50 ppm was approximately 50% higher than control values.

The reproductive data on F_1 dams is presented in Table I. No effects were seen on the parameters listed as a result of HTO exposure or the HTO/Pb interaction. The results do indicate, however, that chronic Pb exposure to 5 ppm increased litter size significantly while exposure to 5 or 50 ppm Pb significantly increased the corpora lutea count and at 50 ppm preimplantation deaths were increased significantly.

Neither HTO, Pb nor the combinations affected F_2 neonatal body weight or the absolute or relative weights of the neonatal liver, kidney, heart or spleen. When pooled across HTO exposures, the 50-ppm Pb lungs weighed about 10% less than controls, although this was not statistically significant.

Table I

Reproductive Performance of F_1 Dams[a]

Pb Level	Litter Size	# Resorptions	Corpora Lutea	Average Pre-Implantation Deaths Per Litter (all litters)	(litters with PID[b])
0 ppm	$(92)^c$ 10.7 ± 0.3[d]	(91) 0.65 ± 0.10	(89) 16.1 ± 0.4	(89) 0.93 ± 0.08	(19) 1.6 ± 0.4
5 ppm	(97) 11.8 ± 0.3[f]	(97) 0.62 ± 0.11	(93) 18.0 ± 0.6[e]	(93) 1.6 ± 0.4	(18) 3.2 ± 0.5
50 ppm	(99) 11.0 ± 0.3	(99) 0.68 ± 0.11	(96) 16.5 ± 0.5[f]	(96) 2.1 ± 0.4[e]	(20) 3.4 ± 0.6[e]

a = No tritium or lead-tritium interaction effects were detectable. Data have been pooled by lead groups.

b = PID = Corpora Lutea - (Live + Resorptions)

c = sample number

d = mean ± SE

e = p<0.05 by ANOVA and t-test

f = p<0.01 by ANOVA and t-test

Table II

Relative % Brain Weight of F_2 Neonates[a]

Treatment	0.0mSi/mI	0.01mSi/dI	0.1mSi/dI	1.0mSi/dI
0 ppm	5.23[b](c)[d] ± 0.09[e]	5.15[b](c) ± 0.08	5.03[b](c) ± 0.10	5.02[b](c) ± 0.05[e]
5 ppm	5.06[b](c)[d] ± 0.09[e]	5.10[b](c) ± 0.09	5.08[b](c) ± 0.08	5.19[b](c) ± 0.08
50 ppm	5.06[b](c)[d] ± 0.05[e]	5.00[b](c) ± 0.04[e]	5.23[b](c) ± 0.07	5.12[b](c) ± 0.08

a = Interaction of HTO and Pb was significant by two-way ANOVA

b = number of litters

c = Mean of litter means ± SE

d = p<0.05 by ANOVA and t-test

e = p<0.01 by ANOVA and t-test

The relative brain weight of F_2 neonates is presented in matrix form in Table II. HTO produced a dose-related trend of reduced mean brain weight which was significant at a dose rate of 300 millirads/day. Pb significantly reduced relative brain weight at both levels whereas combined exposure to HTO and Pb produced an interaction effect which was less than either agent alone.

One of the consequences of chronic Pb exposure is shown in the organ Pb levels in Table III. HTO had no effect on Pb accumulation and all data have been compiled by Pb treatment. As expected, the major repositories of Pb were bone, kidney and liver. Of particular interest were the observations that a) the maternal/neonatal blood Pb ratio was a constant ratio of approximately 1.3 across all Pb groups; b) intrauterine exposures resulted in Pb accumulation in neonatal lung but not brain; c) there appeared to be a sex difference in Pb content of adult organs.

Postnatal brain catecholamine levels are presented in Table IV. The relative brain weight deficits present at birth in the F_2 due to HTO or Pb exposures were not present at 20 or 180 days. Neither HTO, HTO/Pb nor generation affected NE or DA concentrations in whole brain and the results have been combined by Pb exposure for both generations. NE and DA were both affected by 50 ppm; at the earlier age DA was significantly depressed and in the adult the NE significantly increased.

The effects of HTO and/or Pb on CNS development are presented in Tables V-VII. There were no significant differences in data obtained from the F_1 and F_2 generations so the results have been combined. HTO or Pb and the interaction of HTO/Pb tended to delay the development of the reflexes and the age at eye opening. Statistically significant delays occurred with the righting reflex (Table VII) and eye opening (Table VI) at a dose rate as low as 3 millirads/day and with the startle reflex at 300 millirads/day. Righting reflex development was delayed after 5 ppm Pb exposure. In nearly all cases the interactions of HTO and Pb were less than additive in their effect.

Table III

Lead concentrations in organs (µg/100g wet weight)[a]

Pb treatment (ppm)	F_1 Dams			F_2 Neonates			180 day Males (F_1 + F_2)		
	0	5	50	0	5	50	0	5	50
Blood	[illegible][b]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Bone							[illegible]	[illegible]	[illegible]
Kidney	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Liver	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Brain				[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Lung				[illegible]	[illegible]	[illegible]			
Heart				[illegible]	[illegible]	[illegible]			
Spleen				[illegible]	[illegible]	[illegible]			
Testes							[illegible]	[illegible]	[illegible]

a = No effect of tritium exposure. All tritium groups were pooled by Pb treatment.
b = sample number
c = mean ± SE

d = p<0.01.
e = p<0.01.

Table IV

Effects of Pb exposure on NE and DA concentrations in whole rat brain (ng/g)

Pb Level	60 days old		180 days old	
	NE	DA	NE	DA
0 ppm	[illegible][b]	[illegible]	[illegible]	[illegible]
5 ppm	[illegible]	[illegible]	[illegible]	[illegible]
50 ppm	[illegible]	[illegible]	[illegible]	[illegible]

a = male rats; F_1 and F_2 generations combined; tritium data pooled by Pb exposure.
b = sample number
c = mean ± SE
d = p<0.05 by ANOVA and t-test
e = p<0.01 by ANOVA and t-test

Table V

Age at Development of the Startle Response in Rats Exposed to HTO and/or Pb[a,b]

Treatment	0.0uCi/ml	0.01uCi/ml	0.1uCi/ml	1.0uCi/ml
0 ppm	11.0±0.2 (111)[c]	10.0±0.1 (110)	10.0±0.2 (110)	10.0±0.0 (9)
5 ppm	10.0±0.2 (97)	11.0±0.2 (110)	10.0±0.0[d] (110)	10.0±0.1 (111)
50 ppm	10.0±0.2 (110)	10.0±0.2 (97)	10.0±0.2 (110)	10.0±0.1 (110)

Table VI

Age at Eye Opening in Rats Exposed to HTO and/or Pb[a,b]

Treatment	0.0uCi/ml	0.01uCi/ml	0.1uCi/ml	1.0uCi/ml
0 ppm	15.0±0.2 (111)[c]	15.0±0.1 (110)[d]	15.0±0.2 (110)[d]	15.0±0.2 (97)[d]
5 ppm	15.0±0.2 (97)	15.0±0.1 (110)	15.0±0.0[d] (110)	15.0±0.2 (111)
50 ppm	15.0±0.0[d] (110)	15.0±0.2 (110)	15.0±0.2[d] (111)	15.0±0.2 (110)

Table VII

Age at Development of the Righting Reflex in Rats Exposed to HTO and/or Pb[a,b]

Treatment	0.0uCi/ml	0.01uCi/ml	0.1uCi/ml	1.0uCi/ml
0 ppm	17.0±0.2 (111)[c]	18.0±0.0[d] (110)	18.0±0.0[d] (110)	20.0±0.0[d] (9)
5 ppm	18.0±0.0[d] (97)	18.0±0.0[d] (110)	19.0±0.0[d] (111)	18.0±0.0[d] (111)
50 ppm	18.0±0.0[d] (110)	18.0±0.0[d] (97)	18.0±0.0[d] (110)	18.0±0.0[d] (110)

a = Interaction of HTO and Pb was significant by two-way ANOVA

b = Values given are for mean day of development ± SE. Value in parenthesis is the number of F_1 + F_2 litters (1 animal/litter).

c = sample number

d = p<0.05 by ANOVA and t-test

Table VIII

Horizontal Maze Activity of Adult Male Rats Exposed to HTO and/or Pb[a,b] (counts/hour)

Treatment	0.0uCi/ml	0.01uCi/ml	0.1uCi/ml	1.0uCi/ml
0 ppm	269±10	224±20	218±7[c]	177±14[c]
5 ppm	177±15[c]	216±20	205±20	187±12[c]
50 ppm	196±10	177±19[c]	194±17[c]	207±12[c]

a = Interaction of HTO and Pb was significant by two-way ANOVA.

b = 180 days old; F_1 and F_2 generations combined; n = 9 litters per dose group; each litter represented by 1 rats; mean ± S.E.

c = p<0.05 by ANOVA and t-test.

Table IX

Interval Analysis of the Anesthetized Electroencephalogram of F_1 Adult Male Rats Exposed to HTO and/or Pb

EEG ITEM	HTO mCi/ml				Pb ppm		PPb/HTO
	0.0	0.01	0.10	1.0	5	50	50/1.0
	(a)[b]	(a)	(a)	(a)	(a)	(a)	(a)
ZPC/5 sec.	23.8±3.7	24.3±3.0	17.9±2.1	18.8±2.4	18.6±1.8	23.8±3.4	24.8±3.7
% Beta	18.7±3.5	18.8±3.7	18.8±3.1	7.4±1.2	8.3±0.5	18.8±3.7	18.8±3.3
% Alpha	29.3±3.1	29.1±1.7	18.3±1.7	13.3±0.8	13.9±2.3	17.2±2.3	19.8±2.5
% Theta	28.4±4.0	31.8±2.5	27.2±5.1	28.7±5.8	32.8±3.0	31.8±1.9	33.8±3.1
% Delta	33.4±6.3	31.8±5.4	46.8±6.7	49.8±2.0	45.0±5.7	30.8±5.7	31.9±5.8

a = sample number

b = mean ± SE

The effects of the various HTO and/or Pb exposure regimens on the spontaneous locomotor activity of 165-day-old male rats are presented in Table VIII. Again no consistent differences were observed between generations and the data have been combined. The values presented are the mean counts per hour per day for 3 littermates over 3 consecutive days of residence in the mase. A total of 6 litters (F_1 + F_2) represent each dose group. HTO exposure produced a dose-related hypoactivity which was significant at 30 millirads/day. Pb exposure at 5 ppm produced results similar to 300 millirads/day but no statistically significant effect on activity was produced by the highest dose of Pb. In all cases, combined exposure to HTO/Pb was less than the additive effects of either individual agent.

In Table IX the interval analysis of the EEG recordings from adult F_1 males is presented. No interaction between HTO and Pb emerged from a two-way ANOVA of the EEG parameters so that only the values for HTO, Pb and the highest HTO/Pb exposures are given. Zero potential crossover (ZPC) is the total number of waveforms per 5-second epoch. The % δ, θ, α, β represent the relative occurrence of specific frequencies of electrical activity within the 5-second epoch. Although not statistically significant, HTO exposure tended to decrease ZPC rate as did the 5 ppm Pb exposure but 50 ppm Pb and the combined HTO/Pb rates were similar to control levels. The reduction in ZPC rate occurred as a result of decreases in the % abundance of the α and β frequency classes with a corresponding increase in the δ frequency. However, the EEG data from the F_2 rats provided no indication of treatment-related alterations in any parameter of the EEG interval histograms.

Complete gross and histopathologic evaluation of F_1 and F_2 adult male rats, exposed to HTO and/or Pb for approximately 200 days, showed no evidence of pathologic sequelae at the dose levels employed.

DISCUSSION

F_0 rats were pre-treated with Pb for 40 days prior to mating to establish steady-state levels in the major soft-tissue repositories for Pb since, in humans, soft-tissue concentrations of Pb are relatively constant after the second decade of exposure [16]. In rats, the liver and kidney constitute rapid exchange pools for Pb with biological half-lives of approximately 4-6 days [17].

Assuming an average water consumption of 25 ml/day, the total daily Pb intake of rats in this experiment was approximately 0.15-1.5 mg or 0.6-6 mg/kg/day. Human adult daily intake averages 0.4 mg or 0.006 mg/kg/day [18].

On the basis of previous work [3], the HTO activities employed would not be expected to produce effects evident on the F_1 generation at birth.

Similarly, continuous feeding of 64 or 512 ppm Pb in the diet of F_0 rats has been reported to have no effect on F_1 litter size or body weight [19].

Throughout the exposure of the F_1 and F_2 generations no overt signs of acute toxicity were noted with any treatment except for a transient body-weight deficit in F_2 HTO animals from days 0-15 postnatally. Elevated urinary levels of ALA, which is considered to be a sensitive indicator of Pb intoxication [20], were seen in the 50 ppm F_1 adult rats at approximately 90 days of age.

The reproductive performance of the F_1 generation exposed to HTO was consistent with an earlier report [21] which showed that continuous parental exposure to activities up to 1.0 uCi/ml had no effect on litter size, resorptions, pre-implantation deaths or neonatal weight.

The effects of lifetime parental exposure to relatively low levels of Pb on the F_2 has been investigated by Morris et al. [19] and Schroeder and Mitchener [22]. The former found no effects on litter size or neonatal weight at 64 or 512 ppm. Schroeder and Mitchener's study is pertinent to the present investigation. They exposed an F_1 generation of rats from conception to 21 ppm Pb and examined the reproductive effects. However, the manner of presentation of the results makes comparisons with the present study impossible. Nevertheless, we noted a significant increase in F_2 litter size in the 5-ppm group coincident with an increased corpora lutea (CL) count. At 50 ppm, no difference from control was seen in litter size, probably because the increased CL was offset by increased pre-implantation deaths.

The decreased relative brain weight of F_2 neonates produced by parents with lifetime exposures to 300 millirads/day confirms a previously observed HTO effect [21]. With respect to Pb, we observed a dose-independent decrease in the relative brain weight at birth which has not previously been reported for chronic low-level exposures. At later postnatal ages this effect was not observed in any treatment group. No topographical or internal organ anomalies were evident in any dose group at birth.

The blood Pb levels in F_1 mothers and their offspring serve to support Baltrop's suggestion that a maternal-fetal blood Pb equilibrium exists [2]. Interestingly, his study of humans shows an average maternal-fetal blood Pb ratio of 1.36, nearly the same value observed in our rats. The differences in blood and organ Pb levels between adult males and F_1 dams is attributable in part to the greater daily water consumption of the female rat [23].

The results of reflex testing indicate that both Pb and HTO administration delay CNS development. A dose-related delay in the righting reflex was produced by Pb with no observed effect on growth. This suggests that Pb is acting directly on the nervous system. HTO produced a delay in the development of the righting reflex and eye opening at all exposure levels and in the startle response at the 1.0 uCi/ml level.

the transient depression of body weight which occurred during the testing period for F_1 startle reflex and eye opening may have contributed to these results since undernutrition may be a factor in the observed delays [24]. However, these effects had disappeared by day 18 which precedes the development of the righting reflex.

The ANOVA showed a significant interaction between Pb and HTO on reflex development and eye opening, indicating the effects are not additive. By inspection, the interactions are less than additive in all cases.

Locomotor activity in the adult animals was also affected by Pb and/or HTO. The HTO administration produced a dose-related decrease in the total activity whereas Pb exposure produced depressions of activity unrelated to dose. As with the reflex development, the effects on activity produced by combined exposure to Pb and HTO were less than additive.

Since the adult CNS has been shown to be very insensitive to both Pb [25] and ionizing radiation [26], these behavioral differences seen in the adult animals are probably due to the effects of Pb and/or HTO exposure on the CNS either in utero or during early development.

Brain catecholamines are known to be associated with spontaneous locomotor activity [27]. The slight, but statistically significant increase in whole-brain NE concentration and the recovery of DA concentrations in the 50-ppm treated adults was not the expected result for hypoactive animals. An explanation for this apparent conflict is that whole-brain catecholamine assays may mask regional effects which are directly involved in the expressed behavior.

The results of the adult F_1 EEG analyses are compatible with the hypoactivity responses of most treatment groups. High-voltage slow waves are characteristic of both natural and anaesthetized sleep. Thus, the increased δ abundance of the δ waves is suggestive of neuronal synchronization and a "resting" CNS. Unfortunately, the significance of these data and the correlation are open to question because of the inability to reproduce these findings in the F_2 generation.

The objective of this study was to determine if 2 potentially synergistic environmental pollutants would in fact interact and to assess the impact on population exposure standards. For the conditions of our study, we have concluded that combined exposures to HTO and Pb are less than additive in their effect and do not impact on the population exposure standards for either agent. The principal findings of this work were that continuous, low-level exposures to 3 millirads/day of HTO or 5 ppm Pb can produce biologically important consequences to mammals.

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DISCUSSION

C.A. BONDHAUS: Your data on age of onset of eye opening and startle responses suggest an antagonistic effect of HTO and Pb together, which appears to cancel the delay due to either of them alone. But does "loss of additivity" necessarily indicate that either agent "protects" against the other? Perhaps the respective insults simply mask each other in different ways. Could you please comment on any such indications or possible mechanisms?

D.F. CAHILL: We do not think that "loss of additivity" should necessarily be equated with a protective effect of either agent against the other. Since the exposure to HTO or Pb alone were sufficient to produce effects, whereas the combined exposures were less effective than either agent, we could speculate that the low level of insult produced by either was insufficient to provoke a compensatory response but that the combined insults did trigger a compensatory reaction.

V. NISHIWAKI: When we were trying to use demineralized water (heavy water) for the diagnosis of kidney disease 20 - 30 years ago in Japan, we observed that the water metabolism and the size of the water pool as well as the excretion rate of water were affected in cases of disturbance of renal function. Is there any possibility in your experiment that the administration of lead could have affected renal function, and that the water metabolism, the size of the water pool of the body and the excretion rate of tritiated water were changed?

D.F. CAHILL: Experiments by Glover at the University of North Carolina have shown that exposure to high levels of lead, of the order of 1 000 to 10 000 ppm, will produce renal dysfunction with a characteristic occurrence of nuclear inclusion bodies in renal tubular cells. Our histopathologic evaluations of the kidneys of rats exposed to much lower doses of lead showed no evidence of nuclear inclusion bodies. This would imply that renal damage did not occur. However, we did not directly test for renal function.

Our experimental design would not permit us to determine any fluctuations in the body water pool because the animals were on a continuous regimen of tritiated drinking water at a constant specific activity; nor was the urinary excretion rate measured.

R.B. HOLTZMAN: What were the lead concentrations in the animals, and how do they compare with those in man and in unexposed animals?

D.F. CAHILL: The lead concentrations in a number of neonatal and adult organs as a function of the exposure regimen are presented in Table III. With respect to the unexposed animals, the chronically exposed adult rats showed significantly higher lead levels in the major organ repositories even at the 5 ppm exposure level.

The blood lead levels of F_1 dams and F_2 neonatal rats exposed to 50 ppm correspond roughly to the average level of lead in human blood. Since at our lowest exposure level we were providing approximately 100 times the adult human daily intake in terms of mg of lead per kg of body weight, the lead levels in the rat organs were many times those of humans.

E.E. POCHIN: If you test a large number of deviations for statistical significance, some, e.g. 5%, will exceed the $p = 0.05$ level purely by chance. Are your estimates of significance corrected for this?

D.F. CAHILL: If I interpret your question correctly, you are asking whether in a 13-cell matrix we would expect one observation to exceed $p = 0.05$ purely by chance. Yes, we would, but similarly we would also expect that the "significant" observation would occur at random throughout the eleven treatment cells. The clear dose-responses in a number of parameters which we measured would argue against these being chance occurrences. The absence of clear dose-responses to HTO in several cases is probably a function of the extremely low radiation doses employed and the insensitivity of the functional tests for discriminating between the low-level effects.

A one-way ANOVA was applied to the results with only HTO or only lead and a two-way ANOVA to the data from groups exposed to both HTO and lead. This was used to screen from differences between treated and untreated populations. The doses at which significant differences arose were further defined by the t-test.

H. GLUBRECHT: I should like to make a general comment which relates to nearly all the papers presented at this session. I wonder if it is meaningful, while investigating incorporated radionuclides, to make complicated and sometimes very arbitrary calculations in order to obtain dose values in rads. We know that, at least in some cases, the transmutation effect plays a major role as compared to radiation effects. Moreover, even if radiation alone is important, all effects depend on the distribution of the radionuclides in the molecular structure of the cells and organisms and this is true especially in the case of tritium with electrons of about 1 μm range. This is a biochemical problem and therefore cannot be solved by

microdosimetry. In cases where we do not have a fairly homogeneous distribution, I would suggest that we desist from giving absorbed energy dose values and define the experimental situation only by parameters which we know with certainty, such as the specific activity of the radioisotope administered and the chemical and physical parameters of treatment.